1110-215 Gap Junction Channels: Coexpression of Cardiac Connexins Restricts Communication Pathways That Modulate Cell Growth but Not Membrane Potential

Bilal S. Ali, Asif Sewani, Carolina Vasquez, Nazem Akoum, Inayat Momin, Alonso P. Moreno, Krannert Institute of Cardiology, Indianapolis, IN

Background: The resting membrane potential (RMP) and cell growth in NRK cells expressing Cx43 is dependent on electrical coupling between cells, which may allow molecules involved in generation of the RMP to pass from cell to cell. Since Cx45 is abundant during the early stages of mouse heart development and may participate in tissue differentiation,we studied the effect of altering the permeability of gap junction channels by co-expression of Cx43 and Cx45 on cell growth and generation of RMP.
Methods: Vectors containing either –EGFP (Enhanced Green Fluorescent Protein) or EGFP and Cx45 were transfected into Cx43 expressing NRK cells. Resistant clones were grown in DMEM modified media. The RMP of the cells was measured using a single electrode patch clamp technique. Cell growth was determined daily for 4 days in both cell lines by counting cells from sister dishes after trypsinization.
Results: NRK-EGFP-Cx45 demonstrated markedly decreased cell growth after 4 days as compared to NRK-EGFP. (7800±346 (Mean±SE) vs 28883±3758, N = 3, p=0.02 per paired t-test). RMP remained related to the number of cells in a cluster but show no significant difference between cell lines. Isolated cells or those with clusters of less than 3 cells had an RMP of -0.26 mV ±0.3mV. In confluent cells the RMP reached -48.04 ± 1.9 mV (NRK-EGFP) and -48.56 ±1.3 mV (NRK-EGFP-Cx45, p=0.54). 100µm 18α-glycyrrhetinic acid 3β-*O*-hemisuccinate(ACO) exponentially abolished the RMP with time constants of 6.12 ± 0.37 hrs and 6.67 ± 0.79 hrs.
Conclusions: Co-expression of Cx45 and Cx43 results in a marked decrease in cell growth but does not appear to affect the RMP or the kinetics of uncoupling by ACO. Since the combination of connexins yields heteromeric channels with low permeability, our data strongly suggests that the mechanisms maintaining cell growth require larger permeability channels (probably for larger molecules) than the mechanism involved in establishing the RMP. NRK cells appear to be a suitable model for studying gap junction permeability and its relation to the kinetics of growth and generation of RMP. The findings could help to clarify certain aspects of cardiac development.

1110-216 Dynamic Intracardiac Calcium Transition in Very Early-Stage Ebryonic Mouse Heart Tube Regulated by G Protein Coupled Signal Pathway

Fuhua Chen, Meisheng Jiang, Thomas S. Klitzner, James N. Weiss, David Geffen School of Medicine at UCLA, Los Angeles, CA

Background: Beta-adrenergic and uscarinic signaling pathways play important roles in the physiological regulation of heart rate, intracellular Ca transients and cell contraction, mediated by G-protein coupled receptors and their downstream targets. However, the timing with which these receptors become functionally coupled in the embryonic heart is not known. Accordingly, we used dynamic video analysis to investigate the regulation of Ca transients in very early-stage embryonic mouse hearts (E9.5 - E11.5 day old) by both the beta-adrenergic agonist isoproterenol (ISO), and muscarinic receptor agonist carbachol (CCh).
Methods: After washing in Tyrode's solution, isolated embryo hearts were incubated for 20 min with 10 µM fluo 3-acetoxymethyl ester, and then transferred to an experimental chamber on an inverted fluorescence microscope containing 1.8 mM Ca Tyrode's solution. Ca fluorescence was imaged using a CCD camera-based video-capture imaging system with appropriate filters for fluo-3.
Results: We found that the spontaneous cardiac Ca transient rate averaged 42±3 beats per min (bpm) (mean±SEM, n=31) at room temperature for in the E9.5 hearts, which was similar to the E10.5 (44±2, n=45) and E11.5 (45±3, n=25) hearts. Exposure to ISO (10 µM) significantly increased the Ca transient rate in all 3 groups by 32% (E9.5, n=28), 69% (E10.5, n=41) and 73% (E11.5, n=24), respectively. However, perfusion of CCh (10 µM) with ISO decreased the ISO-augmented Ca transient only in the E10.5 (from 75±2 to 64±3 bpm, n=40, p<0.001) and E11.5 (from 79±4 to 41±3 bpm, n=23, p<0.001) hearts, but not in E9.5 (from 57±2 to 56±1 bpm, n=23, p=NS) hearts.
Conclusion: These data show that functional beta-adrenergic signaling precedes functional muscarinic signaling during mouse cardiac embryogenesis.

1110-217 Autologous Fibroblast Transplantation Into Myocardial Infarcts in Pigs: Effects on Arrhythmogenesis and Arrhythmic Threshold

Fernando Tondato, Keith Robinson, Jianhua Cui, James Sanzo, Traci Goodchild, Melissa Fowlkes, Randall Lee, Mark Maciejevski, Nicolas Chronos, Nicholas Peters, American Cardiovascular Research Institute, Atlanta, GA

Background: Ventricular myocardial dysfunction is associated with lethal arrhythmias, secondary to non-uniformity of myocardial electrophysiology and abnormal conduction pattern. Autologous cell transplant has potential to either worsen or suppress arrhythmic tendency depending on effects on electrophysiological heterogeneity. Autologous fibroblasts (Afbr) are a realistic option for cell therapy, being readily available, easily cultured, and resistant to hypoxia. Afbr may modify arrhythmic tendency by creating conduction block in infarcted regions, and can readily be transduced to modify phenotype and membrane channel expression. Objective: To test whether Afbr transplant to infarcted myocardium modifies arrhythmogenesis. Material and Methods: Eight pigs were subjected to myocardial infarction by coronary occlusion with embolized microspheres. A skin sample was harvested for dermal Afbr isolation, culture and tagging with BrdU. At 3 wk, animals (randomized) received epicardial injections of either tagged dermal Afbr or medium alone via thoracotomy. A total of 2.5 ± 1ml (68 ± 43 x 10^6 cells/ml) of solution was injected, before which electrophysiologic study (EPS) with programmed stimulation (2 RV sites, drive cycle length = 500, 400 and 350ms, up to 3 extra-stimuli) was performed, and a subcutaneous wireless ECG-telemetry system implanted. Continuous ECG was recorded for 4 wk, after which repeat EPS was performed and animals were sacrificed for evaluation of infarcts by histopathology and BrdU immunostaining. Results: Immunostaining revealed engraftment of tagged cells in infarction scar. ECG analysis showed reduction in number of episodes of non-sustained VT (0.07±0.37 vs 30.6±148.2 episodes/day; P<0.01) and a reduced total number of beats of non-sustained VT (0.7±3.3 vs 199.8±976.8: P<0.01) in the Afbr group compared with sham.At 4-wk EPS, 2 control and none of the Afbr animals were more inducible of VT than at baseline. Conclusion: Dermal Afbr engraft in ischemic myocardium and are associated with a reduction in arrhythmic episodes and arrhythmogenicity. These initial findings indicating antiarrhythmic properties of Afbr transplantation warrant further investigation.

1110-218 The Slope of the Restitution Curve Is Steeper on the Epicardium Than Transmurally

Jian Huang, Hao Qin, Raymond E. Ideker, University of Alabama at Birmingham, Birmingham, AL

The restitution hypothesis is that conduction block occurs with a restitution slope >1 and arrhythmogenicity increases as the slope increases beyond 1. It has recently been shown that early during ventricular fibrillation (VF) the epicardium activates faster than the remainder of the ventricular wall suggesting that it is the region most responsible for the maintenance of VF. Therefore, we tested the hypothesis that the maximum slope of the restitution curve is greater epicardially than transmurally.
We recorded from 1056 electrodes, spaced 2 mm apart, in 6 pigs. A 24x21 electrode plaque was sutured on the left anterior and lateral epicardium. Two orthogonal rows of plunge needles were inserted to form a cross through the middle of the plaque with 24 needles in the horizontal line and 21 needles in the vertical line. Each needle contained 12 electrodes, 1 mm apart, with the first electrode 1 mm beneath the epicardium and the twelfth electrode near the endocardium. We calculated activation recovery intervals (ARIs) for the last of 30 cycles paced at a constant cycle length. Cycle lengths spanning the interval from 400 to 130 ms were used. ARIs were defined as the time between the maximum negative dV/dt of the last QRS complex and the maximum positive dV/dt of the following T wave. Restitution curves for each of the 1056 electrodes were fit as a sigmoid function.
The mean epicardial ARI at a pacing cycle length of 400 ms was significantly longer (218±28 ms) than the mean ARI of all the transmural needle electrodes (207±27 ms). However, accommodation was greater at the epicardium so that at a cycle length of 130 ms the mean epicardial ARI (97±11 ms) was not significantly different than transmurally (98±10 ms). The maximum slope of the restitution curve was >1 throughout the myocardium with a significantly greater value at the epicardium (4.2±0.9) than transmurally (3.4±1.3). In conclusion, consistent with the restitution hypothesis, we found that the maximum slope of the restitution curve throughout the pig ventricular wall is >1 and the largest slope is in the epicardium, which has previously been reported to be the fastest activating region during early VF.

1110-219 Regionally Altered Action Potential Duration Restitution in Swine With Hibernating Myocardium and Sudden Cardiac Death

Michael D. Banas, Gen Suzuki, James A. Fallavollita, John M. Canty, Jr., University at Buffalo, Buffalo, NY, VA Medical Center, Buffalo, NY

Background: Pigs with hibernating myocardium develop SCD from ventricular fibrillation (VF). We tested the hypothesis that this was secondary to regionally altered APD restitution.
Methods: We compared APD restitution in pigs (n=9) instrumented with a chronic LAD stenosis to produce hibernating myocardium with sham controls (n=6) in the closed-chest propofol-sedated state. Bipolar LV pacing and LV monophasic action potentials (MAP) recorded from sites in the hibernating LAD region were compared to sham animals as well as normally perfused posterobasal regions in the same heart. The LV was paced at a basic cycle length of 500 ms and APD_{70} restitution curves were created using an S1-S2 protocol. Single premature stimuli were delivered after every 10^{th} beat as the coupling interval was decremented by 2-10 ms until the ventricle was refractory.
Results: Typical restitution curves are shown below. The slope of the lower portion of the APD restitution curve exceeded unity in hibernating myocardium and was significantly greater than shams (1.66 ± 0.23 vs. 0.92 ± 0.02, p<0.05). The restitution slope of normally perfused remote myocardium was similar to that of sham controls.
Conclusion: These data support the notion that APD restitution is regionally altered in hibernating myocardium. This may contribute to the predisposition to spontaneous ventricular fibrillation and increased mortality observed in swine as well as humans with hibernating myocardium.